ELSEVIER

Journal of Cystic Fibrosis 11 (2012) 63 – 67

Journal of Cystic Fibrosis

www.elsevier.com/locate/jcf

Short Communication

Ancestral haplotype 8.1 and lung disease severity in European cystic fibrosis patients☆

Harriet Corvol [a, b, *, 1], Julie Beucher [a, 1], Pierre-Yves Boëlle [b, c], Pierre-François Busson [c], Céline Muselet-Charlier [a], Annick Clement [a, b], Felix Ratjen [d], Hartmut Grasemann [d], Judith Laki [e], Colin N.A. Palmer [f], J. Stuart Elborn [g], Anil Mehta [e]

[a] AP-HP, Hôpital Trousseau, Pediatric Pulmonary Department; Inserm U938, Paris, France
[b] Université Pierre et Marie Curie-Paris 6, Paris, France
[c] AP-HP, Hôpital St Antoine, Biostatistics Department, Inserm UMR-S707, Paris, France
[d] Division of Respiratory Medicine, Department of Pediatrics, the Hospital for Sick Children, and University of Toronto, Toronto, Canada
[e] Division of Medical Sciences, University of Dundee, Dundee, United Kingdom
[f] Biomedical Research Institute, University of Dundee, Ninewells Hospital and Medical School, Dundee, DD1 9SY, United Kingdom
[g] Respiratory Medicine Group, Queen's University of Belfast, Belfast, United Kingdom

Received 10 July 2011; received in revised form 12 September 2011; accepted 17 September 2011
Available online 10 October 2011

Abstract

Background: The clinical course of cystic fibrosis (CF) lung disease varies between patients bearing identical *CFTR* mutations. This suggests that additional genetic modifiers may contribute to the pulmonary phenotype. The highly conserved ancestral haplotype 8.1 (8.1AH), carried by up to one quarter of Caucasians, comprises linked gene polymorphisms on chromosome 6 that play a key role in the inflammatory response: *LTA +252A/G; TNF −308G/A, HSP70-2 +1267A/G* and *RAGE −429T/C*. As inflammation is a key component inducing CF lung damage, we investigated whether the 8.1AH represents a lung function modifier in CF.
Methods: We analyzed the lung function of 404 European CF patients from France ($n=230$), Germany ($n=95$) and UK ($n=79$). FEV_1 differences between 8.1AH carriers and non-carriers were calculated in each country and pooled using a random effects model.
Results: The frequency of 8.1AH carriers was similar between French (22%), German (29%) and UK (27%) patients. We found that 8.1AH carriers had significantly lower FEV_1, adjusted for age classes and countries ($P<0.04$, mean FEV_1 difference −6.4% CI95% [−12.4%, −0.5%]). No difference was observed with respect to BMI *Z*-scores and chronic colonization with *P. aeruginosa*.
Conclusions: These findings support the concept that 8.1AH is an important genetic modifier of lung disease in CF. To conclude, multiple linked genes outside the CF locus might explain some of the variability in lung phenotype.

Keywords: Cystic fibrosis; 8.1 Ancestral Haplotype; Modifier genes; Lung function

1. Introduction

Cystic fibrosis (CF) is a common inherited disease that gradually destroys the lung and is found mainly in people of European descent [1]. Compelling data on phenotypic variability and the lack of genotype-phenotype correlation among either siblings or unrelated patients with identical mutations in the *cystic fibrosis transmembrane conductance regulator (CFTR)* gene has led to

☆ These data have been presented in conferences: NACFC 2010—Baltimore (Pediatric Pulmonology 2010, supplement 33: abstract page 282) and ECFC 2011—Hamburg (invited talk—genetics symposium).

* Corresponding author at: Hôpital Trousseau, Pediatric Pulmonary Department, 26, avenue du Dr Netter; 75012 Paris, France. Tel.: +33 1 44 73 61 74; fax: +33 1 44 73 67 18.
E-mail address: harriet.corvol@trs.aphp.fr (H. Corvol).

[1] Equally contributed.

1569-1993/$ - see front matter © 2011 European Cystic Fibrosis Society. Published by Elsevier B.V. All rights reserved.
doi:10.1016/j.jcf.2011.09.006

the suggestion that gene modifiers may affect the lung phenotype [2,3]. However, the problem with the studies which have tried to associate the severity of CF lung disease with modifier genes, for example those involved in the innate or acquired immune response, is that candidate polymorphisms have a very low prevalence in the community at large. Thus it either follows that CF phenotypic variability is a mosaic of different rare modifiers or that some highly prevalent but unknown co-inherited factors are at work. With respect to the first idea, replicated results suggest that single-nucleotide polymorphisms (SNPs) in *transforming growth factor B1* (*TGFB1*) [4–6] and *mannose binding lectin 2* (*MBL2*) [7,8] genes might explain a small percentage of the observed phenotypic variability. Here, we test the second idea that differential co-inheritance of a latent but highly prevalent cassette of linked genes (haplotypes) might underpin the observed variability in lung function. The term haplotype refers to the block structure of a genetic region and haplotype differences mean that the discrepancies may arise from differences in distribution of more than one allele in combination with others. Some haplotypes have a high prevalence in Europe and we wondered if one of these could contribute to disease variability in large numbers of CF patients.

The highly conserved ancestral haplotype 8.1 (8.1AH) is encoded in the major histocompatibility complex (MHC) region on the short arm of chromosome 6 [9]. 8.1AH appeared to be a good candidate because it is carried by up to one quarter of Caucasians and comprises a cassette of linked alleles that play key roles in the inflammatory response: *LTA +252A/G* (Lymphotoxin A), *TNF−308G/A* (Tumor necrosis factor), *HSP70-2* +1267A/G (Heat shock protein) and *RAGE*−429T/C (Receptor for Avanced Glycation Endproducts). Moreover, 8.1AH has been associated with delayed onset of lung bacterial colonization in CF patients in a small cohort of Hungarian patients [10]. As airway inflammation is a key component inducing CF lung damage, we investigated whether the 8.1AH represents such modifier in European CF patients from France, UK and Germany.

2. Methods

The study population consisted of a total of 404 European CF patients (adults and children), enrolled from 6 CF centers in France (230 children); one CF center in Germany (95 children) and one CF center in UK (79 adults). The study was approved by the ethical committees of the Medical Review Board of each participating CF center in France, Germany and UK; and written informed consent was obtained for each patient. The diagnosis of CF was made on the basis of two abnormal sweat chloride test results (>60 mmol/L) and/or identification of *CF-causing* mutations [11]. Clinical, biological and functional data were obtained from hospital records from the previous 2 to 5 years in France, Germany and UK. The data were gathered by a single physician for each country, blinded to the results of patient's haplotypes. Recorded data included date of birth, sex, *CFTR* genotype, pulmonary function tests, nutritional status and airways microbiology. Lung function was assessed by spirometry in children >6 years during periods of clinical stability. Respiratory microbial flora was determined by microscopy and culture of lower respiratory tract secretions or throat swabs realized every 3 months in all the CF centers. Chronic airway colonization with *Pseudomonas aeruginosa* (*P. aeruginosa*) was defined by the persistence of the pathogen in at least three airway samples for at least 6 months. Nutritional status was appreciated by the *Z*-score for the body mass index (BMI).

Genotyping were performed by real time polymerase chain reaction (PCR), using a 2700 thermocycler and the conventional Taqman primers and probes (Applied Biosystems, Foster City, USA). Allelic discrimination was realized by endpoint measurements with specific fluorescent oligonucleotides (detection system software of the ABI prism 7000). We typed 4 linked polymorphisms of the 8.1AH: *LTA* +252A/G (rs909253, NC_000006.11:g.31540313A>G), *TNF*−308G/A (rs1800629, NC_000006.11:g.31543031G>A), *HSP70-2* +1267A/G (rs1061581, NC_000006.11:g.31784586G>A) and *RAGE*−429T/C (rs1800625, NC_000006.11:g.32152442A>G).

Haplotypes were reconstructed using the EM algorithm, using genotypes at the 4 linked polymorphisms [12]. As influence of 8.1 AH carriage on phenotype has been described either with the whole haplotype, i.e. carriage of the 4 variants simultaneously, or with part of its constituents, i.e. 3 out of the 4 variants, we defined 8.1AH when at least one haplotype included at least 3 variants among: *LTA +252G*, *TNF−308A*, *HSP70-2* +1267G and *RAGE*−429C [9,13]. The association in 8.1AH carriage and age was tested using Cochran–Armitage test for trend. BMI *Z*-scores were calculated using the WHO 2007 growth reference [14]. Colonization at age 18 was calculated using the Kaplan–Meier method and compared using the log-rank test stratified on country. Forced expiratory volume in 1 second (FEV_1) was standardized for age and sex by computing percent-predicted relative to reference Knudson equations [15,16]. Mean percent-predicted FEV_1 differences between 8.1AH carriers and others were calculated in each country and age class and pooled using a random effects model. Heterogeneity between FEV_1 differences in the country/age class subgroups was tested using Cochran's *Q* statistic. The same analysis was done for BMI *Z*-scores and CF specific centiles *Z*-scores.

All analyses were made using the R software.

3. Results

The clinical characteristics of the study population are listed in Table 1. Median current age was 11.3 years for the French, 16 years for the German and 26.5 years for the UK patients. Most of the individuals were pancreatic insufficient (93%) and about half were female. Similar to the distribution of *CFTR* mutations in CF European patients, 56% were homozygous for the p.Phe508del *CFTR* mutation and 34% p.Phe508del compound heterozygous. Distribution of the 8.1AH carriers was similar to that observed in Caucasians [9] and similar across the 3 populations: 22% in the French; 29% in the German CF and 27% in the UK patients (Chi-squared test=2.4; $P=0.30$). The frequency

Table 1
Clinical characteristics of the study population.

	France	Germany	UK
Study population, *n*	230	95	79
Current age : median [range]	11 [6–18]	16 [6–18]	26.5 [23–54]
Sex, female / male	108/122	39/56	36/43
CFTR genotype, %			
F508del homozygous	52%	62%	61%
F508del compound heterozygous	37%	35%	24%
Other mutations	11%	3%	15%
Pancreatic insufficiency, %	92%	98%	91%
BMI *Z*-score : median [range]	−0.49 [−3.33;3.80]	−0.63 [−4.54;1.24]	−0.37 [−2.77;3.26]
P. aeruginosa colonization at age 18, % [95% CI]	51% [41%–63%]	43% [33%–57%]	82% [74%–91%]
8.1AH carriers	22%	29%	27%

of 8.1AH carriers did not change markedly with age (trend test, $P=0.18$).

FEV_1% predicted was smaller in 8.1AH carriers in each country and age class except in UK patients aged [26–30], with little heterogeneity among age classes and countries (Cochran's Q statistic for heterogeneity, $P=0.99$).The random-effect pooled difference showed that 8.1AH carriers had significantly lower FEV_1% predicted overall ($P<0.04$, mean FEV_1 percent predicted difference, carriers vs. non carriers −6.4% CI95% [−12.4%, −0.5%], Fig. 1).

Differences in BMI *Z*-scores according to 8.1 AH carriage were not always in the same direction, fluctuating around 0 (Fig. 2); Cochran's Q statistic for heterogeneity, $P=0.20$). The pooled measure did not reveal any difference in nutritional status according to 8.1AH carriage ($P=0.76$). Finally, chronic colonization with *P. aeruginosa* was not different according to ancestral carriage ($P=0.99$).

4. Discussion

The key novel finding of the present study is that co-inheritance of a widely prevalent ancestral haplotype 8.1AH is associated with a greater lung disease severity in CF manifesting as a significantly lower FEV_1 across the 3 European populations including children and adults. Our data also suggest that in modifier gene studies, genetic associations at the haplotype level may be more informative rather than focusing on individual SNPs [17]. Moreover, our data are consistent with the current understanding of the roles of 8.1AH in the inflammatory response. 8.1AH has indeed been associated with excessive and uncontrolled inflammation in various inflammatory disorders [18,19]. In CF, inflammation is believed to drive the progressive destruction of the lung and the decline in lung function which is responsible for the major morbidity and mortality [20]. 8.1AH comprises linked polymorphisms of major pro-inflammatory cytokines including LTA, TNF, HSP and RAGE. TNFα and LTA are 2 key members of the TNF superfamily involved in the inflammatory response. HSP70-2 protects other proteins against aggregation and mediates the folding of newly translated proteins. In CF, protein misfolding is thought to contribute to disease pathogenesis [21]. RAGE is a member of the immunoglobulin superfamily, highly expressed in pulmonary tissues and acts as a pro-inflammatory mediator *via* MAP kinases and NF_kB pathways activation which are both abnormal in CF [22–24]. Moreover, neutrophils from CF airways have been shown to express increased levels of RAGE [25]. The potential involvement of this haplotype in CF phenotypic differences is consistent with the early onset, excessive and persistent airway inflammation that is due partly to an imbalance between pro- and anti-inflammatory cytokines in CF.

Critically, had we followed the classical single polymorphism approach, we would have found no association between individual *LTA, TNF, HSP* and *RAGE* single SNPs and lung function in our European cohort (data not shown) and yet we

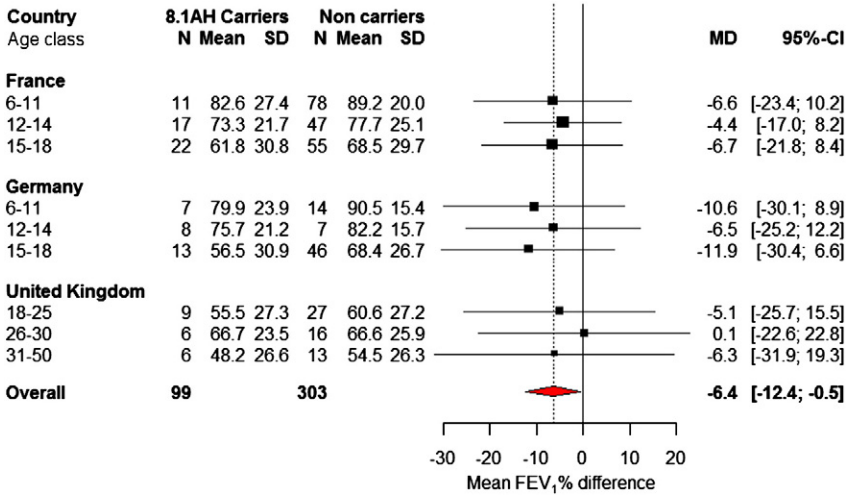

Fig. 1. Ancestral haplotype 8.1 AH carriage and FEV_1 in European patients with cystic fibrosis. FEV_1 differences were calculated in each country and age class and are shown as black squares with 95% confidence interval (segments). A random-effects pooled estimate of the FEV_1 difference according to 8.1AH carriage is shown as the bottom diamond, centered on the mean difference and extending to the limits of the pooled 95% confidence interval. On average, FEV_1 was significantly lower in 8.1 AH carriers ($P<0.04$).

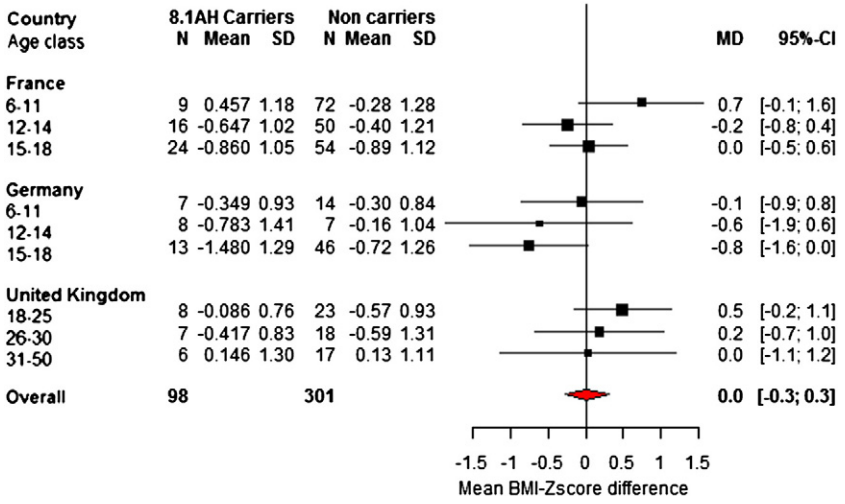

Fig. 2. Ancestral haplotype 8.1 AH carriage and Body Mass Index in European patients with cystic fibrosis. BMI *Z*-score differences were calculated in each age class and country and are shown as black squares with 95% confidence interval (segments). A random-effects pooled estimate of the BMI *Z*-score difference according to 8.1AH carriage is shown as the bottom diamond, centered on the mean difference and extending to the limits of the 95% confidence interval. There was no difference between 8.1 AH carriers and non carriers ($P=0.76$).

found a significant association with the 8.1AH. The *TNF −308G/A* genotype has been previously explored as potential lung disease modifier in different CF populations, but conflicting results have been shown so far [5,6,26–28]. An association between haplotypes including *TNF* variants with CF lung severity was first proposed by Yarden et al. [29]. They studied 3 variants in the *TNF* promoter (−851C/T, −308G/A, −238G/A) and one in the *LTA* 1st intron (+691Gins/del) in 180 European p.Phe508del homozygous CF children (58 from Belgium and 122 from the Czech Republic). They found no association for *TNF*−308G/A or *TNF*−238G/A but patients heterozygous for *LTA* +691Gins/Gdel were more likely to have a better lung function compared to patients homozygous for *LTA* +691Gins; furthermore they observed a higher proportion of *TNF*−851C carriers in the group of patients with an FEV_1 below 70% predicted. These results led them to suggest that associations between the *TNF* gene and CF phenotypes could involve haplotypes rather than single genotypes. The interpretation of single locus associations has indeed several limitations, and there is evidence that in a complex disease such as CF, analyses based on haplotypes can provide additional power in detecting significant association [4].

In CF, association between 8.1AH and lung disease has been previously explored by Laki et al. in 72 CF patients, 39 of which were p.Phe508del homozygote and 33 p.Phe508del compound heterozygous [10]. They showed that 8.1AH was associated with delayed onset of respiratory colonization with *Staphylococcus aureus* and *P. aeruginosa*, two major pathogens associated with lung disease severity in CF. They concluded that the excessive inflammatory response associated with the 8.1AH may influence the defense system efficiency against some microorganisms. Another recent study also suggests a protective effect of 8.1AH carriage in a healthy population with severe pulmonary infection [30]. Several alterations of the immune response have also been shown to be correlated to AH 8.1, and even immunoglobulin (Ig) levels seem to be influenced by this haplotype [31,32]. Interestingly, Candore et al. have shown that 8.1AH carriers had decreased IgG2 serum levels, which might decrease bacterial clearance and favor chronic colonization [33]. Larger populations of CF patients of European origin are now being assembled, that will help study this issue further [34]. For example, it might be that an elevated inflammatory response is beneficial in the early stages of childhood CF but ultimately becomes destructive as chronic infection ensues in older patients.

In summary, our findings support the concept that the very common 8.1AH that is randomly co-inherited with defects in *CFTR*, appears to be an important genetic modifier of lung disease in CF in up to one quarter of patients. Interestingly, this highly conserved ancestral haplotype contains a cassette of linked genes that play a key role in the inflammatory response which together could be involved in the excessive airway inflammation in CF.

Funding sources

Institut National de la Santé et de la Recherche Médicale, Assistance Publique-Hôpitaux de Paris, Université Pierre et Marie Curie Paris, Agence Nationale de la Recherche, DGS, Association Vaincre La Mucoviscidose, Chancellerie des Universités (Legs Poix), Association Agir Informer Contre la Mucoviscidose, GIS-Institut des Maladies Rares. JB was supported by Rennes CHU-Hospital. JL was supported by a grant from Tayside R&D awarded to AM in Dundee.

Conflict of interest statement

None of the authors have any commercial or other associations that might pose a conflict of interest.

Acknowledgements

The authors would like to acknowledge the families and the patients for their participation. We would also like to thank the numerous health care providers and community clinics for their support and participation. Finally, we would like to especially thank Pr Jacques Brouard Dr. François Bremont, Dr. Bertrand Delaisi, Pr. Jean-François Duhamel, Pr. Christophe Marguet, and Pr. Michel Roussey for all of their effort towards the study.

References

[1] O'Sullivan BP, Freedman SD. Cystic fibrosis. Lancet May 30 2009;373 (9678):1891–904.
[2] Stanke F, Becker T, Kumar V, et al. Genes that determine immunology and inflammation modify the basic defect of impaired ion conductance in cystic fibrosis epithelia. J Med Genet Jan 2011;48(1):24–31.
[3] Vanscoy LL, Blackman SM, Collaco JM, et al. Heritability of lung disease severity in cystic fibrosis. Am J Respir Crit Care Med May 15 2007;175 (10):1036–43.
[4] Bremer LA, Blackman SM, Vanscoy LL, et al. Interaction between a novel TGFB1 haplotype and CFTR genotype is associated with improved lung function in cystic fibrosis. Hum Mol Genet Jul 15 2008;17(14): 2228–37.
[5] Corvol H, Boelle PY, Brouard J, et al. Genetic variations in inflammatory mediators influence lung disease progression in cystic fibrosis. Pediatr Pulmonol Dec 2008;43(12):1224–32.
[6] Drumm ML, Konstan MW, Schluchter MD, et al. Genetic modifiers of lung disease in cystic fibrosis. N Engl J Med Oct 6 2005;353(14):1443–53.
[7] Garred P, Pressler T, Madsen HO, et al. Association of mannose-binding lectin gene heterogeneity with severity of lung disease and survival in cystic fibrosis. J Clin Invest 1999;104(4):431–7.
[8] McDougal KE, Green DM, Vanscoy LL, et al. Use of a modeling framework to evaluate the effect of a modifier gene (MBL2) on variation in cystic fibrosis. Eur J Hum Genet Jun 2010;18(6):680–4.
[9] Kiszel P, Kovacs M, Szalai C, et al. Frequency of carriers of 8.1 ancestral haplotype and its fragments in two Caucasian populations. Immunol Invest 2007;36(3):307–19.
[10] Laki J, Laki I, Nemeth K, et al. The 8.1 ancestral MHC haplotype is associated with delayed onset of colonization in cystic fibrosis. Int Immunol Nov 2006;18(11):1585–90.
[11] Farrell PM, Rosenstein BJ, White TB, et al. Guidelines for diagnosis of cystic fibrosis in newborns through older adults: Cystic Fibrosis Foundation consensus report. J Pediatr Aug 2008;153(2):S4–S14.
[12] Excoffier L, Slatkin M. Maximum-likelihood estimation of molecular haplotype frequencies in a diploid population. Mol Biol Evol Sep 1995;12(5): 921–7.
[13] Laki J, Kiszel P, Vatay A, et al. The HLA 8.1 ancestral haplotype is strongly linked to the C allele of -429T>C promoter polymorphism of receptor of the advanced glycation endproduct (RAGE) gene. Haplotype-independent association of the -429C allele with high hemoglobinA1C levels in diabetic patients. Mol Immunol Jan 2007;44(4):648–55.
[14] de Onis M, Onyango AW, Borghi E, Siyam A, Nishida C, Siekmann J. Development of a WHO growth reference for school-aged children and adolescents. Bull World Health Organ Sep 2007;85(9):660–7.
[15] Knudson RJ, Lebowitz MD, Holberg CJ, Burrows B. Changes in the normal maximal expiratory flow–volume curve with growth and aging. Am Rev Respir Dis Jun 1983;127(6):725–34.
[16] Rosenfeld M, Pepe MS, Longton G, Emerson J, FitzSimmons S, Morgan W. Effect of choice of reference equation on analysis of pulmonary function in cystic fibrosis patients. Pediatr Pulmonol Mar 2001;31(3):227–37.
[17] Stanke F, Becker T, Cuppens H, et al. The TNFalpha receptor TNFRSF1A and genes encoding the amiloride-sensitive sodium channel ENaC as modulators in cystic fibrosis. Hum Genet Apr 2006;119(3):331–43.
[18] Candore G, Modica MA, Lio D, et al. Pathogenesis of autoimmune diseases associated with 8.1 ancestral haplotype: a genetically determined defect of C4 influences immunological parameters of healthy carriers of the haplotype. Biomed Pharmacother Sep 2003;57(7):274–7.
[19] Toth EK, Kocsis J, Madaras B, et al. The 8.1 ancestral MHC haplotype is strongly associated with colorectal cancer risk. Int J Cancer Oct 15 2007;121(8):1744–8.
[20] Regamey N, Jeffery PK, Alton EW, Bush A, Davies JC. Airway remodelling and its relationship to inflammation in cystic fibrosis. Thorax Jul 2011;66(7):624–9.
[21] Luciani A, Villella VR, Esposito S, et al. Defective CFTR induces aggresome formation and lung inflammation in cystic fibrosis through ROS-mediated autophagy inhibition. Nat Cell Biol Sep 2010;12(9):863–75.
[22] Hunter MJ, Treharne KJ, Winter AK, Cassidy DM, Land S, Mehta A. Expression of wild-type CFTR suppresses NF-kappaB-driven inflammatory signalling. PLoS One 2010;5(7):e11598.
[23] Muselet-Charlier C, Roque T, Boncoeur E, et al. Enhanced IL-1beta-induced IL-8 production in cystic fibrosis lung epithelial cells is dependent of both mitogen-activated protein kinases and NF-kappaB signaling. Biochem Biophys Res Commun Jun 1 2007;357(2):402–7.
[24] Vij N, Mazur S, Zeitlin PL. CFTR is a negative regulator of NFkappaB mediated innate immune response. PLoS One 2009;4(2):e4664.
[25] Makam M, Diaz D, Laval J, et al. Activation of critical, host-induced, metabolic and stress pathways marks neutrophil entry into cystic fibrosis lungs. Proc Natl Acad Sci U S A Apr 7 2009;106(14):5779–83.
[26] Arkwright PD, Pravica V, Geraghty PJ, et al. End-organ dysfunction in cystic fibrosis: association with angiotensin I converting enzyme and cytokine gene polymorphisms. Am J Respir Crit Care Med Feb 1 2003;167(3): 384–9.
[27] Hull J, Thomson AH. Contribution of genetic factors other than CFTR to disease severity in cystic fibrosis. Thorax 1998;53(12):1018–21.
[28] Schmitt-Grohe S, Stuber F, Book M, et al. TNF-alpha promoter polymorphism in relation to TNF-alpha production and clinical status in cystic fibrosis. Lung Mar-Apr 2006;184(2):99–104.
[29] Yarden J, Radojkovic D, De Boeck K, et al. Association of tumour necrosis factor alpha variants with the CF pulmonary phenotype. Thorax Apr 2005;60(4):320–5.
[30] Aladzsity I, Madach K, Szilagyi A, et al. Analysis of the 8.1 ancestral MHC haplotype in severe, pneumonia-related sepsis. Clin Immunol Jun 2011;139(3):282–9.
[31] Modica MA, Freddi S, Caruso C. Blood IgA, IgM and IgE levels are influenced by sex and HLA phenotype. Exp Clin Immunogenet 1989;6(4): 251–7.
[32] Mohammadi J, Ramanujam R, Jarefors S, et al. IgA deficiency and the MHC: assessment of relative risk and microheterogeneity within the HLA A1 B8, DR3 (8.1) haplotype. J Clin Immunol Jan 2011;30(1): 138–43.
[33] Candore G, Campagna AM, Cuppari I, Di Carlo D, Mineo C, Caruso C. Genetic control of immune response in carriers of the 8.1 ancestral haplotype: correlation with levels of IgG subclasses: its relevance in the pathogenesis of autoimmune diseases. Ann N Y Acad Sci Sep 2007;1110: 151–8.
[34] McCormick J, Mehta G, Olesen HV, Viviani L, Macek Jr M, Mehta A. Comparative demographics of the European cystic fibrosis population: a cross-sectional database analysis. Lancet Mar 20 2010;375(9719): 1007–13.